APPENDIX I

Standard curve for D-glucose

[Graph showing the relationship between O.D. at 575 nm (µm) and Amount of D-glucose (µg).]
APPENDIX II

PREPARATION OF THE REAGENTS FOR THE ESTIMATION OF CELLULASE ACTIVITY

1. Dinitrosalicylic acid reagent (DNS)

See Appendix – VI.

2. Citrate Buffer

\[
\begin{align*}
A &= 0.05 \text{ M Citric acid solution (5.252 g in 500 ml)} \\
B &= 0.05 \text{ M Sodium citrate solution (7.352 g in 500 ml)}
\end{align*}
\]

'x' ml of A + 'y' of B, diluted to a total 100 ml with distilled water.

<table>
<thead>
<tr>
<th>x ml</th>
<th>y ml</th>
<th>pH</th>
</tr>
</thead>
<tbody>
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<td>3.5</td>
<td>3.0</td>
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<td>43.7</td>
<td>6.3</td>
<td>3.2</td>
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<tr>
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<td>10.0</td>
<td>3.4</td>
</tr>
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<td>37.0</td>
<td>13.0</td>
<td>3.6</td>
</tr>
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<td>35.0</td>
<td>15.0</td>
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<td>28.0</td>
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</tr>
<tr>
<td>7.2</td>
<td>42.8</td>
<td>6.2</td>
</tr>
</tbody>
</table>

3. 2 % Carboxymethyl Cellulose (CMC)

2 g CMC was added to 100 ml citrate buffer and warmed at 40°C in oven for 30 min.

4. Roschelle Salt

20 g od Sodium-Potassium-Tartarate was dissolved in small amount of distilled water and final volume was made to 100 ml.
APPENDIX III

PROTOCOL FOR THE ESTIMATION OF CELLULASE ACTIVITY
(Miller, 1959; Mandels et al., 1976)

EXPERIMENT
2 ml 2% CMC
(in 0.05 M Citrate buffer, pH 4.8)
pH 4.8
1 ml crude enzyme extract

\[\rightarrow\]

3 ml DNS reagents was added to each set

\[\rightarrow\]

Kept on boiling water bath for 5 min.

\[\rightarrow\]

Cooled rapidly

\[\rightarrow\]

1 ml Roschelle salt solution was added to each set before it cooled down

\[\rightarrow\]

\(A_{575}\)

CONTROL

2 ml 2% CMC
(in 0.05 M Citrate buffer,

1 ml heat killed crude enzyme extract

Incubated at 50\(^\circ\)C for 30 min.

Blank: Instead of Crude enzyme extract, 1 ml sterile distilled water was added with CMC.
APPENDIX IV

ESTIMATION OF CELLULASE ACTIVITY IN TERMS OF IU/ml

Concentration = \frac{1}{\text{dilution}} = \frac{\text{volume of enzyme sample in dilution}}{\text{total volume of dilution}}

The m.w. of glucose is 180, hence, 1 \mu \text{ mol glucose} = 0.18 \text{ gms.}

0.5 \text{ mg glucose released} = \frac{1}{0.18 \times \text{conc. of enzyme} \times \text{time}}

0.5 \text{ mg glucose} = \frac{1}{0.18 \times 1 \times 30} \mu \text{mol} / \text{min} / \text{ml}

= 0.185 \mu \text{mol} / \text{min} / \text{ml}

\text{CMCase (IU/ml)} = \frac{0.185}{\text{enzyme concentration required to release 0.5 mg glucose}}

During the CMCase assay, 1 ml of enzyme in 1 ml buffer was used. Hence the amount of enzyme required to release 0.5 mg glucose will be (A)

= \frac{0.5}{\text{Amount of glucose (mg) released by 1 ml of enzyme used}}

\text{IU/ml} = \frac{0.185}{A}

[1 IU = 1 \mu \text{ mol of hydrolysis product (glucose) released per min.}]
APPENDIX V

Standard Curve for D-xylose
Stock solution of D-xylose with concentration 2 \mu g/ml D.W
APPENDIX VI

Preparation of the reagents for the estimation of xylanase activity

1. Dinitrosalicylic acid reagent (D.N.S.) (Miller 1959) for measuring reducing sugars. Protocol (to make 1 litre).

2% NaOH is dissolved in 400 ml water (D.W.) in an Erlenmeyer aluminium foiled flask

↓

Add 8 g Phenol crystal stored at 40°C temperature.

↓

Add 10 g D. N.S.

↓

Keep on magnetic stirrer without heating

↓

When D.N.S. dissolve completely, add Roschelle salt 200g (slowly-2).

↓

After dissolving it, add Sodium Sulphite 2 g.

↓

Make all contents as 1 litre by adding D.W.

2. Potassium Phosphate Buffer

A = 0.1 M \( K_2 HPO_4 \) (17.418 g/l) 278 ml

B = 0.1 M \( KH_2 PO_4 \) (13.609 g/l) 722 ml

Mix volume A and B to make one litre Buffer and adjust pH to 6.0.
APPENDIX VII

PROTOCOL FOR THE ESTIMATION OF XYLANASE ACTIVITY

(Miller et al., 1959)

I. Xylan = 10mg ml

II. A. 1.6 ml enzyme + 0.4 ml xylan

B. 1.6 ml enzyme + 0.4 ml (0.1M) Potassium Phosphate Buffer (pH 6.0)

C. 1.6 ml Buffer + 0.4 ml xylan.

↓

Incubate at 55°C for 15 min.

↓

Centrifuge A and C for 1 min. in Apandroph's tube.

↓

Pellet discarded

Supernatant (S.N.)

↓

1.0 ml S.N. of A and C and 1.0 ml from B.

↓

3 ml D.N.S. is added separately in each 1 ml volume taken from A, B and C, respectively.

↓

Keep in boiling water bath for exactly 5 min.

↓

Cool under running water.

↓

Record absorbance at 575 n.m.
APPENDIX VIII

SOME FORMULAE USED FOR THE ESTIMATION OF XYLANASE

Xylanase activity was calculated using the following formula:

\[
\text{IU/ml} = \frac{\text{xylose released (mg/ml) \times \frac{1}{\text{time of incubation (in min)}} \times \frac{1}{\text{vol. of enzyme used (ml)}}}}{\frac{1}{150.1} (\text{mol. vol. of xylose}) \times \text{volume of total Assay \times dilution}}
\]

\[
= \frac{\text{ug/ml xylose released \times \frac{2.0 \text{ ml}}{3602.1 \times \text{dilution}}}}{\text{IU/ml}}
\]
APPENDIX IX

Standard curve for Protein Assay (BIO-RAD Reagent)